# A STUDY OF PEDAL LACERATION IN ACTINIANS.

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Since the appearance of Andres's ('82) classical paper "Intorno alla Scissiparita della Attinæ," the only accounts of the process of pedal laceration have been very casual, or have been given only for the sake of comparison with the phenomena of induced regeneration. Carlgren ('04), ('09), who alone has studied this form of reproduction in the genus *Aiptasia*, makes mention only of the sequence of the appearance of the mesenteries and tentacles, but gives no general account of the process, nor does he mention any of the histological features.

Since the last-mentioned phase of the subject is of considerable interest as a comparison with the development of normal embryos, it has seemed advisable to publish the following account to supplement the previous contributions to the knowledge of this type of reproduction which is so common among the actinozoa.

The observations herein recorded are based upon the study of four species of actinians: *Aiptasia pallida* Agg., *A. tagetes* D. & M., *A. annulata* Andres and *Cylista leucolena* Agg.[1] Of these species *A. pallida* and *Cylista* were obtained in abundance at Beaufort, N. C.,[2] during the summer of 1903 and 1904, where they were studied both in their natural environment and in aquaria in the laboratory.

Specimens of the first-mentioned species were transferred to Baltimore and kept during two years in aquaria in the zoölogical laboratory of the Johns Hopkins University. By means of the diatom method described by Dr. Caswell Grave for rearing echinoderm larvæ, the actinians were kept in good condition

[1] Professor McMurrich informs me that he has conclusive evidence that the form described by Verrill from Beaufort, N. C., as *C. leucolena* is not the *C. leucolena*, of A. Agassiz, but in the absence of any published statement to that effect Verrill's terminology will be used.

[2] I am indebted to the officials of the U. S. Bureau of Fisheries for the use of a table, and equipment at their Beaufort, N. C., laboratory during the two seasons above mentioned.

during this time and they continued to reproduce by laceration throughout the year, although no embryos were ever set free during either of the breeding seasons.

The specimens of *A. tagetes* studied were obtained in 1909, during a stay at the Bermuda Biological Station.

*A. annulata* was obtained during the past summer while the writer was at the laboratory of the Carnegie Institution of Washington at Dry Tortugas, Florida.

The two last-mentioned forms were studied in aquaria in the laboratory only.

Material of the four species was preserved, after being narcotized in magnesium sulfate, in a number of fixing reagents; of which Petrunkewitsch's fluid and Bouin's fluid gave the best results. All of the *Aiptasias* cut readily after being imbedded in paraffin in the usual manner, while the mesoglea of the *Cylistas* proved so refractory that double imbedding in celloidin and paraffin was necessary in order to secure an unbroken series of sections.

In common with the tissues of most actinians, many of the hæmatoxylin stains were highly selective, staining the ectoderm much more heavily that the endoderm so that by using an alcoholic solution of eosin as a counter stain after Delafield's hæmatoxylin there resulted an absolutely certain color differentiation between the two tissues by means of which any question of the origin of a certain structure could be determined by its staining reaction.

## General Account of the Process of Laceration.

As has been described by Andres (1882), the beginning of the process of laceration is characterized by a certain part of the base of an actinian becoming very firmly attached to the substratum while the animal as a whole moves away from the point which is thus relatively immovably attached. As a result the tissues about this point become strongly stretched and attenuated. With the continuation of the contraction, there finally comes a rupture of the tissues at some little distance from the free border of the disc so that the resulting laceration piece has usually an appearance such as is shown in Fig. 1, Plate I., which represents a fragment from the base of a specimen of *A. pallida* immediately after its separation from the parent individual. The *acontia*, which in laceration pieces of this form are at first so prominent, are later either withdrawn inside the cavity of the fragment, or else they are broken off before the open side of the piece has closed to any considerable extent.

Immediately after the separation of the fragment there takes place a rolling-in of the free edges, due principally to the elasticity of the mesoglea, so that for some little distance within the cavity is lined with ectoderm (Fig. 7, Pl. II.). For some hours after the separation of the fragment there is no visible external change other than an increasing loss of color about the orifice of the tear where the laceration piece was separated from the parent individual.

The next noticeable change appears as a turning upward of that portion of the fragment in which is situated the original opening, which by this time has become markedly reduced in extent; although, contrary to the results of Hazen (1902), who worked with fragments obtained by cutting off pieces including portions of the column wall and pedal disc from adult specimens of *Sagartia lucæ*, in none of my specimens did the opening become entirely obliterated. The new growth which brings about this change in the shape of a laceration piece is much the more rapid on the lower-pedal disc-side of the aperture. So that for some time the elevated area is situated near the former internal edge of the fragment. The wall that has come from the up-growth from the pedal disc is nearly perpendicular, while the opposite side of the fragment slopes away much as in its former manner (Fig. 2, Pl. I.).

In a stage of development such as is represented in Fig. 2 there has already appeared the beginning of the tentacle buds, and internally, of course, the earliest mesenteries.

In a later stage, as in Fig. 3, Pl. I., the readjustment of materials had proceeded farther in what is practically the same course. The fragment as a whole has become much higher. The oral end has nearly the same diameter, while farther down toward the pedal disc the diameter is relatively much decreased. At the base, however, the fragment still covers practically the same area as when it was torn off from the parent animal. When viewed from the side, as in this figure, the lines corresponding to the insertion of the old mesenteries are seen to extend only a short distance upward from the base and to be less distinct toward that side which is most nearly perpendicular, *i. e.*, which has arisen from the tissue which was originally part of the pedal disc. The tentacle buds have increased very slightly in size, but at the oral end of the fragment, which now has the appearance of a partly contracted *Aiptasia* there can be made out through the almost transparent column wall, the outlines of the stomodeum and the lines of insertion of the developing mesenteries.

Figs. 4 and 5 were, in order to show the arrangements of the tentacles, drawn in a nearly oral view, so that the upper part of the column is not visible. The fact that the lines of insertion of the old mesenteries appear to extend nearly the whole length of the column is thus to be explained by the foreshortening in the figure and not by any difference as regards the mesenteries between these specimens and that shown in Fig. 3. In the specimen shown in Fig. 4, there are eight tentacles, of which one is considerably longer than the other seven, all of which are of approximately the same length. In practically all instances one tentacle outstrips the others in its development so that for a considerable time it may be distinguished from the others. On the other hand there seems to be no such definiteness in the determination of just what tentacle shall first appear as there is in many actinian embryos where the tentacle above the endocœle between the two dorsal directive mesenteries almost invariably makes its appearance first.

In later stages there is often a very marked difference in the sizes of the tentacles of the first series, as in the specimen represented in Fig. 5. Here a single tentacle has far outstripped the others, while of the remaining seven three are decidedly larger than the remaining four. In Fig. 5 the tentacles of the second set are seen to be arising in pairs at each side of several of those of the first set. While that tentacle of the first set which is uppermost in the figure is by far the longest, there is on either side of it a single short tenacle of the first set, and no indication of the paired tentacles of the second series. The tentacles on the opposite side of the disk have already acquired their definite shape and appearance.

The young actinian shown in Fig. 6 has already acquired the characteristic appearance of all small specimens of this species, so that, from an external examination, it would be impossible to determine whether it had come directly from an egg or from a laceration piece. As compared with any of the figures of earlier stages the proportionate diameter of the column is very much reduced. This has been brought about by a continuation of the same processes that are shown at an earlier stage in Fig. 3. The basal portion of the column is now of no greater diameter than the upper part, while the whole animal has become considerably elongated. The first eight tentacles are now of practically the same length as are also the eight pairs of secondary tentacles. Internally the stomodeum can be seen to have increased greatly in length. The mesenteric filaments, not previously distinguishable, have now become very prominent structures on the first eight mesenteries. The column wall has become thin and has lost its pigment clear to the base through which are seen the points of insertion of the first eight mesenteries, while those mesenteries that came over from the parent actinian in the laceration piece, have entirely disappeared, even at the extreme base of the young specimen.

In following through the development of any single laceration piece from the time of its separation from the parent until it becomes a typical young actinian it is apparent that there has been very little, if any, increase in its bulk, and that the change in shape has come about almost entirely through the readjustment of the materials already present and not to any considerable extent through the metabolism of new material.

*Frequency of Reproduction by Laceration among the Forms Studied.*

The following table shows the frequency of this method of reproduction among the four species studied and the number of young that has arisen from a single parent individual within a given time.

In another instance many thousand individuals of *A. pallida* were examined casually, but without any records being kept, and here again it was found that the percentage of those which had undergone laceration was very high. In fact this form of reproduction was much more frequent in the last-mentioned instance—on the government jetties at Cameron, La., than in the case of the same species from which the records were made at Beaufort.

| Species. | Number Examined. | Number that had Given Rise to Laceration Pieces. | Number of Laceration Pieces. | Locality. |
|---|---|---|---|---|
| *C. leucolena* ....... | 300 | 292 | 11.32 (0–30) | Beaufort, 1903. |
| *C. leucolena* ....... | 1,000 | 942 | | Beaufort, 1904. |
| *A. pallida* ........ | 150 | 78 | 6 (0–14) | Beaufort, 1904. |
| *A. tagetes* ......... | 220 | 187 | 10.4 (0–26) | Bermuda, 1909. |
| *A. annulata* ....... | 52 | 20 | 3 (0–7) | Tortugas, 1910. |

## Internal Changes During Laceration.

Under this head will be discussed the following topics:

(*a*) The formation of the stomodeum.

(*b*) The development of the mesenteries.

(*c*) The development of the mesenteric filaments and acontia.

(*d*) General histological changes in the tissues during development.

### (*a*) *The Formation of the Stomodeum.*

As mentioned previously in the general account of the process of laceration, the first noticeable change in the appearance of a laceration piece after its separation from the parent consists in the rolling in of the edges of the torn side of the fragment, so that from the first the opening into the cavity of the fragment is lined for some distance with ectoderm (Fig. 7, Pl. II.). At first the ectoderm extends forward for only a short distance on the upper side of the opening, while on the lower side the inrolling has progressed considerably farther.

In a section through a stage such as is shown in Fig. 2, Pl. I., the stomodeum has the appearance such as is shown in Fig. 8, Pl. II. Here the free edges about the orifice of the fragment have become thinner, the mesoglea is reduced to a thin sheet of tissue and the cavity of the stomodeum has become vertical instead of nearly horizontal as at first. At the lower, free, border of the stomodeum the ectoderm has become turned outward and extends around the border of the endoderm onto the internal wall of the stomodeum.

In Fig. 9, Pl. II., which represents a section through a stage slightly older than that shown in Fig. 3, Pl. I., the stomodeum has reached nearly its normal length and, on the left side in the figure, its ectodermal lining is shown extending down along the border of a mesentery to form the mesenteric filament. On the opposite side where the section passes through the chamber between mesenteries, the ectoderm forms a flat plate extending across the free border of the stomodeum.

### (*b*) *The Development of the Mesenteries.*

Andres (*loc. cit.*), in his figures illustrating the rearrangement of the mesenteries in the young fragments, shows only the pedal disc and apparently understood that the mesenteries which come over in the laceration piece from the parent were rearranged to become the permanant mesenteries of the young actinian. He makes no mention of any new growth of mesenteries or stomodeum at the oral end of the fragment as it becomes elongated and assumes the characteristic shape of an actinian.

Carlgren, 1904, 1909, takes up to a considerable length the question of the arrangement of the mesenteries in laceration embryos. He distinguishes four types of arrangement of the first twelve mesenteries in such embryos: depending both on the sequence in the appearance of the mesenteries, and on whether or not any of these mesenteries, are situated in old tissue. "Was erstens die Entwicklungstypus der Mesenterien in den Lacerationstückchen anbelangt, so haben die 1904 und die hier oben mit geteilten Untersuchungen gezeugt dass es verschiedene Variationen derselben giebt. Wie gross die Variation auch ist, so kann mann doch deutlischerweise in dem Lacerationstückchen, d. h. in solchen Stückchen, die einen Teil der Fussscheibe und einen der Korperwand umfassen (Text Fig. 1, *b*), drei Verschiedene Haupttypen für die Mesenterien Entstehung unterscheiden, und zwar erstens einen *bilateralen* mit *drei* primären, vollständigen, gleich, orientierten Mesenterienpaaren (II., 4.), Zweitens ein *bilateralen* mit nur *zwei* ebenfalls gleich (II., 1), angeordneten Paaren und schliesslich drittens einen *birädialen* (II., 5 and 8), welche letzteren zwei Neubildungszonen enthält, die in ihren am besten entwickelten Gestalt (No. 8), Spiegelbilde zu einander sind, indem jede aus zwei bilateral angeordneten, vollständigen Mesenterienpaaren besteht." Besides these three types Carlgren recognizes a mixed type which cannot be put in any one of the above-mentioned classes.

All the specimens of *Aiptasia* studied by Carlgren fell, with three exceptions which were very irregular in their growth, into his group three, the biradial type, in which there are two zones of new growth. All of the specimens of this genus that I have sectioned show this arrangement of the newly formed mesenteries, which, thus, seems to be characteristic of the genus *Aiptasia*.

The specimens of *Cylista* also showed the same arrangement of the first mesenteries.

H. V. Wilson, 1888, states that in *Macina areolata* the œsophageal invagination is at first centrally placed in the larva, but that soon "it begins to travel toward one side of the larva." This lateral motion of the stomodeum continues until that organ comes to lie close against the outer wall of the larva. Finally as the lateral movement progresses—from above downward—the endoderm is pushed before it until the two ectodermal layers, of the stomodeum and of the body wall, are in contact, or separated only by the thin layer of mesoglea secreted between them. Soon following this stage, . . . "the œsophagus has moved away from the surface ectoderm, but while doing so has remained connected with it by a band of supporting lamella." The mesentery arises by the endoderm growing up into the space between the stomodeum and the outer wall of ectoderm as the stomodeum is drawn toward the side opposite the first mesentery, for the formation of the second one.

Appellöf, 1900, mentions the same sort of displacement of the stomodeum previous to the formation of the first mesentery. He states, on the other hand, that: "Die exzentrische Lage des Schlundrohres ist in allen Entstehungsphasen desselben konstant und deutlich nachzuweisen und ist—so nehme ich wenigstens an—auf ungleichseitiges Wachstum der Larve zurückzuführen."

In laceration embryos the stomodeum is from its first appearance excentric in position. As the change in the form of the piece takes place so that the original orifice, where the piece was torn off from the parent, becomes carried up toward the highest part of the fragment and becomes smaller and more rounded, the stomodeum on that side which has come from the growth of the pedal disc comes into contact with the body wall. The first mesentery makes its appearance at the point where the forming stomodeum is in contact with the column wall; although in none of my sections is there evidence of any marked displacement of the endoderm such as would be necessary to bring the two mesogleal surfaces into contact. As is always true in the formation of a mesentery, the growth of the mesogleal lamella here begins at the extreme oral surface and proceeds downward along the stomodeum and column wall. In laceration embryos the endoderm is carried down on both sides of the mesogleal lamella so that from its earliest appearance, the mesentery is structurally complete. As the growth leading to the acquisition of the characteristic actinian shape of the laceration piece goes on the stomodeum becomes situated more centrally in the oral disc. While this adjustment is taking place the first mesentery becomes lengthened in the horizontal direction to keep pace with the change in the position of the stomodeum.

The method of the formation of all those mesenteries formed after the stomodeum has become central in position is the same. The first indication of their appearance is seen as a slight ridge of mesoglea extending aborally from the oral disc at the point where the latter joins the column wall. As the growth of the mesentery continues the mesogleal lamella extends farther from its point of origin, both horizontally along the oral disc and proximally along the column wall. When by its horizontal growth the mesogleal lamella comes in contact with the mesoglea of the stomodeum a rapid downgrowth of the mesentery takes place. Throughout the development of a mesentery the growth is the most rapid where there is contact between the growing mesogleal lamella and that of the body wall, oral disc, column wall or pedal disc. In all such mesenteries the growth of the endoderm keeps pace with that of the mesogleal lamella so that the latter never extends to the free border of the mesentery.

In Figs. 10 to 14, Pls. II. and III., are shown a number of sections through the same laceration embryo at different levels. In Fig. 10, which passes close to the oral disc, only four complete mesenteries are present while a member of the third pair has made its appearance on one side of the longitudinal axis of the stomodeum.

In Fig. 11, a section taken through the embryo near the base of the stomodeum, seven mesenteries are present. The same four as noted in the previous section are complete while both those of the third pair are present and one of the fourth pair is seen as a slight prominence of the endoderm containing a very thin mesogleal fold. In Fig. 12 only six mesenteries are present, but it seems that one of the third pair is lacking instead of the single one of the fourth pair which was seen in the previous section.

The first pair have already acquired their mesenteric filaments, which at this stage extend only a short distance below the stomodeum.

Farther down, at the level of the section shown in Fig. 13, only four new mesenteries are present, while seven of those which came over in the laceration piece from the parent are seen. This last-mentioned section was taken about one third of the distance from the oral disc to the base of the embryo, and in this series the newly formed mesenteries are found in only four of the following sections.

In the sections shown in Fig. 14, all of the mesenteries present are those which were in the laceration piece at the time when it was separated from the parent. Fig. 14, although taken at some distance from the base shows, in comparison with the previous sections, that the basal portion of the embryo is of considerably greater diameter than the more distal portions.

A comparison of the three last-mentioned figures illustrates one phase of the redistribution of materials in the growth of a laceration embryo. In Fig. 13, that side where the new mesenteries are being formed is very thin-walled, the mesoglea having become reduced to a thin sheet throughout that half of the body. In the remaining half, on the other hand, the tissue relations of an adult *Aiptasia* are still maintained. In the sections through the more proximal part of the body the adult tissue relations are apparently undisturbed, at least morphologically. It will be seen, however, that in this region there has been coincident with the elevation of the oral end of the embryo a drawing together of the basal portion so that it is now nearly circular in outline. In Fig. 15 it may be seen that the old mesenteries which formerly occupied only one side of the fragment—at the time of its separation from the parent—have now come to be distributed so that they now arise from about four fifths of the circumference.

In sections through an older specimen, as shown in the series of figures from Fig. 15 to Fig. 18, the second set of mesenteries has made its appearance about the inner surface of the oral disc, and eighteen of them have reached the stomodeum. Farther down along the stomodeum (Fig. 17), the twelve primary mesenteries are complete while only one other pair has made sufficient downward growth to appear in such a section.

In Fig. 18, where eleven of the twelve primary mesenteries are present, none of those which came over in the laceration piece from the parent animal are in evidence. Eight of the new mesenteries have acquired their filaments, but only those on the first pair of mesenteries are trilobed.

As a basis for the comparison of this series with the previous one attention may be called to the appearance of the mesoglea in Fig. 18. At a corresponding height on the column of the individual shown in the former series (Fig. 14), none of the newly formed mesenteries were present, while in Fig. 13, where only four of them had appeared, that side of the column on which they were situated showed all of the characteristics of newly formed tissue.

This shows beyond question that in the older specimen the new mesenteries have extended down to a part of the body where there were formerly some of the older mesenteries which by this time have been entirely resorbed. It will be noticed that in this part of the body the tissue readjustment has proceeded until the relation between the layers characteristic for the adult has been reached.

Carlgren, 1904, in discussing a series of sections through an embryo in which eight of the new mesenteries had reached the stomodeum figures two sections and makes statements from which I can infer only that he understands, either that some of the old mesenteries are brought into the permanent systen of mesenteries of the laceration embryo, or that some of the new mesenteries arise at a level below the lower end of the stomodeum. Either of these conclusions is at variance with the results which I have obtained in the study of the four species of actinians on which the present paper is based.

Carlgren's figures and the statements directly relating to them are as follows:

Ein querschnitt in der Schlundrohrsregion (Fig. 4, Taf. IX.) zeigt ein birädial Anordnung der vollständigen Mesenterien mit den beiden Enden des spaltförmigen Schlundrohrs sind zwei Richtungsmesenterienpaare vereinigt. An jede Seite derselben liegen zwei vollständige Mesenterien (*a*) ich nenne sie später kürzlich seitliche Mesenterien—die ihre Längsmuskelen gegen einander kehren. In allem sind also 8 Mesenterien vollständig.

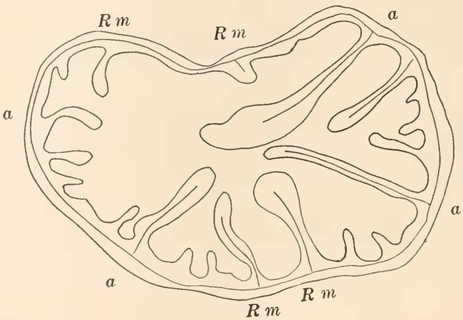


FIG. 1. (Carlgren's Fig. 4, Taf. IX.)

(Wenn ich unter von einer birädialen Anordnung sprache, sind die vollstandigen Mesenterien wie hier grupiert.) Zwischen den Seitlichen Mesenterien an jeder Seite sind verscheidene, unvollständige Mesenterien entstanden, die in dem proximal körpertheilen (Fig. 7, Taf. IX.), ziemlich staak sind; in den distalen an der einen Seite verschwinden, an der anderen nur unbedeutend entwickelt sind oder ganz fehlend (Fig. 4, Taf. IX.). Die mit (*b*) bezeichneten Mesenterien bilden mit ihren Partneren an je ein Mesenterienpaar mit zugewandten Langsmuskeln. Die Mesenterien des Stückchens sind also nach der Sechszahlangeordenet, obgleich von dem ersten Cyklus vier Mesenterien unvollständig sind. Die zwischen den Mesenterien (*b*), liegenden

Mesenterien gehören einem zweiten Cyklus an. An jeder Seite der Richtungsmesenterien findet man auch unvollständige Mesenterien eines zweiten Cyklus. Auch einige Mesenterien der dritten Ordnung sind angedeutet (Fig. 7, Taf. IX.). An

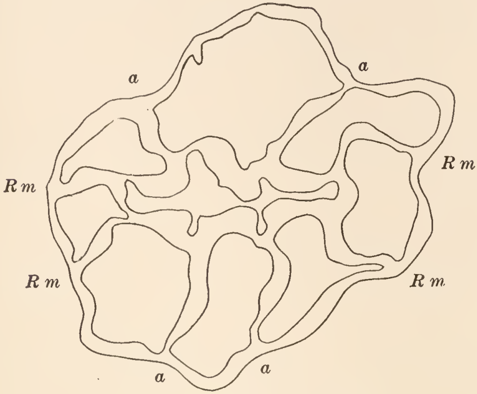


Fig. 2. (Carlgren's Fig. 7, Taf. IX.)

der Bases sind die mit (*a*) bezeichneten Mesenterien am Stärksten, die Mesenterien (*a*) an der rechten Seite der Sagittalachse laufen fast mit den entsprechenden Mesenterien an der anderen zusammen (Fig. 7, Taf. IX.).

His Fig. 4, Taf. IX., my text Fig. 2, shows a condition of the mesenteries which is common to all laceration embryos in a corresponding stage of development. His Fig. 7, Taf. IX., text Fig. 3, shows twenty-five mesenteries, eight of which he considers to be of the first cycle (mesenteries marked *a*) while of the remaining seventeen six are according to his statement of the second cycle (marked *b*) and the remainder of a third cycle. It will be noticed that several of the mesenteries in this figure—one pair of those marked *Rm* and the longest ones at one end of the figure—are very broad in section with a thick mass of endoderm extending far beyond the mesoglea. None of the entire number of mesenteries have any trace of mesenteric filaments, nor by their structure, in so far as it can be made out from the original figures, is there any indication that they contain actively growing tissue as is always found in the new mesenteries in the forms that I have studied. Comparing these two figures with those of the series shown in my Figs. 10 to 14, Pls. II. and III., it seems very evident that all of the mesenteries shown in Carlgren's Fig. 7, Taf. IX., are old ones which have come over in the fragment from the parent individual and which will never come to be a part of the permanent system of mesenteries of the actinian arising from the laceration embryo. In the younger embryo shown in my series of figures just mentioned the first formed pair of mesenteries has already acquired their mesenteric filaments, which are as yet, of course, in an undifferentiated condition; but nevertheless clearly distinguishable as mesenteric filaments.

In the embryo from which my series of Figs. 16 to 18 was taken, which has at the level of the stomodeum only four more mesenteries than that from which Carlgren's figures under discussion were taken, eight of the new mesenteries, at the level at which Fig. 18 was taken, are provided with filaments of which those on the first pair of mesenteries are already trilobed, while several of the others show the first steps in the process of differentiation by which the trilobed condition is reached.

### (*c*) *The Development of the Mesenteric Filaments.*

The question of the origin of the mesenteric filaments among the anthozoa has been discussed to considerable length by most workers on the development of these forms. With the exception of E. B. Wilson, 1884, all of the previous investigators have confined themselves to a consideration of the embryonic development. The above-named investigatór worked with the polyps arising by budding in several alcyonarians, but not with embryos of the same forms.

E. B. Wilson (*loc. cit.*) reached the conclusion that in the Alcyonaria the dorsal directive filaments arise as downgrowths of the ectoderm of the stomodeum, while all the other filaments arise through the differentiation of the endoderm at the free border of the mesentery. He would homologize the dorsal filaments of the Alcyonaria with the lateral ciliated bands (flimmerstriefen) of the trilobed actinian filament. The filaments of the remaining mesenteries he would homologize with the glandular streak (Nesseldrüsenstreif) of the trilobed filament. Thus, according to his interpretation, the actinian filament is a double structure.

H. V. Wilson (*loc. cit.*) working on the coral *Manicina*, where the filament consists of a single lobe, reached the conclusion that this type of filament was comparable to the trilobed actinian filament and is entirely of ectodermic origin.

McMurrich, 1891, describes a double origin for the trilobed filaments of *Rhodactis* and *Aulactinia;* after the manner suggested by E. B. Wilson.

Duerden, 1899, working on *Lebrunia*, where the mesenteries become developed before the stomodeum becomes hollowed out, concluded that in this case part, at least, of the filaments are of endodermic origin since he finds stages where filaments are present on some of the mesenteries which as yet have no direct connection with the stomodeum.

Appellöf (*loc. cit.*) concludes that the mesenteric filaments are developed entirely from the ectodermal downgrowth from the lining of the stomodeum. He mentions having observed a number of instances where the ectodermal layer had become very much attenuated just below the level of the stomodeum, and indeed in some instances the continuity of this layer had been broken. He argues from this fact that some of the instances observed by previous workers who, on the basis of a lack of continuity between the mesenteric filament and the lining of the stomodeum, maintained the endodermic origin of the filaments might be simply cases where the continuity of the tissue had been broken and in no way fundamentally different from the ordinary course of events as he has described them. He also makes the suggestion that the method of development described by E. B. Wilson for the alcyonarian filaments may be confined to those individuals which arise as buds and not a true explanation of what takes place during embryonic development, which he tacitly assumes would be similar to plans followed in other Anthozoan forms.

In all descriptions of the formation of the mesenteries, and particularly of the mesenteric filaments, except that of Duerden (*loc. cit.*) for *Lebrunia*, it is recorded that the filaments appear first on the upper part of the mesenteries at the level of the free border of the stomodeum, and usually there can be observed a direct continuity between the ectodermal lining of the stomodeum and the filaments from the first appearance of the latter.

The mesenteries acquire their filaments in the same order as that in which they arose, so that the dorso-laterals would first show these structures. In Fig. 12, Pl. II., this pair of mesenteries alone shows the beginning of the filaments. Farther up in this series of sections the tissue forming the filaments is directly continued into the lining of the stomodeum. In Fig. 9, Pl. II., a longitudinal section through a young laceration embryo, the filament extends for a short distance down along the free border of the mesentery, and here there is no histological differentiation to mark the lower limits of the stomodeum. Its actual limit can, however, be determined by a comparison with the other side of the figure where the section passed between two mesenteries so that the border of the stomodeum hangs free in the gastrocœl. H. V. Wilson (*loc. cit.*) has pointed out that in *Manicina* the filaments of all the first twelve mesenteries, save the first pair, arise from a fold of the ectoderm at the free border of the stomodeum which bends up and outward into the gastrocœl pointing toward the oral disc. In this manner the filament begins to grow down the mesentery before the edge of the latter has in its upper part come into contact with the stomodeum.

In the development of laceration pieces of *Aiptasia* there is the same folding backward of the wall of the stomodeum at an early stage in the development (Fig. 8), so that the course of the development of the filaments in this form follows that already described for *Manicina*.

In an older specimen, Fig. 18, Pl. II., the filaments of the first pair of mesenteries have assumed the trilobed form characteristic of the filaments of adult *Aiptasias*, while the remainder of the mesenteries have at this level either simple filaments or none at all. It is thus apparent that the change in the structure of the filaments follows the same order as does the appearance of the filaments themselves.

In this transition from the simple to the tri-lobed structure there is in these forms no indication of the origin of the ciliated bands as an acquisition of new or different tissues, as McMurrich has suggested in arguing for a dual origin for the parts of the complex filament, but, on the other hand, the lateral lobes of the complex filament arise by a very slight differentiation of the cells already present in the filament from the time of its origin from the stomodeal ectoderm. In Fig. 17, Pl. III., the filaments of the dorsal directive, the ventro-lateral and the ventral directive mesenteries all show in their centers the beginning of the growth which will give to the mesogleal layer its characteristic branched appearance. Each of these branches will become the center and support, of one lobe of the adult trefoil filament. In the dorsal directive mesenteries especially, it may be noticed that the tissues of the filament have extended out around the edges of the mesentery so that the filament is nearly circular in outline. Originally the extension of the stomodeal ectoderm—first appearance of the filament—was in the form of a flat sheet (Fig. 12, Pl. II.). The separation of this sheet of tissue into the three lobes of the adult filament apparently takes place through an unequal growth from three points in the nearly circular filament, as shown in Fig. 18, Pl. III.

As may be seen by comparing Fig. 9 and Fig. 12, Pl. II., with the fully formed filament (*e. g.*, the filaments of the dorso-lateral mesenteries in Fig. 17, Pl. III.) the histological changes which have taken place in the transformation of the "embryonic" tissue of the stomodeal ectoderm to those of the tri-lobed filament have been in the nature of a separation of the cellular elements of the original tissue so that one type of cells (ciliated) is centralized in the lateral lobes; while the other types (gland and nettle cells) are concentrated in the median lobe. The apparent change is greatest in the case of the median lobe, since the undifferentiated stomodeal ectoderm is at the time of the first appearance of the filaments poor in gland cells and nematocysts. This last-mentioned fact is also true of the activity growing ectoderm of the new part of the column wall and oral disc, and in all parts of the body there is a marked increase in the actual as well as in the proportionate number of both these types of cellular elements after the normal body proportions have been established.

(*d*) *Histological Changes During Development by Laceration.*

In considering the histological changes undergone by the tissues during development by pedal laceration those changes which take place in the basal portions of the parent individual before the fragment is torn off may be first mentioned. Andres (*loc. cit.*) mentions that in *Aiptasia lacerata* the rim of the basal portion of the animal becomes very opaque, and that in section there seems to be an unusual number of gland cells in this region. The endoderm becomes very much thickened in this region so that when the fragment becomes torn off there is only a small cavity left in its interior (Fig. 7, Pl. II.). As may be seen in this section, the thickening of the endoderm is very much greater on the upper side of the fragment, *i. e.*, that which has come from the original column well.

In the period immediately following upon the separation of the fragment from the parent, there is a rapid change in the character of the tissues about the orifice. All of the newly forming tissue becomes very light in color due to the comparative paucity of the zoöxanthellæ in the endoderm of the active area. The mesoglea becomes comparatively thin in this region, but seems always to arise as a direct prolongation of that already present and not as a new secretion between the ectoderm and endoderm of the new parts.

In the cellular layers the changes are more profound. In the ectoderm (Fig. 19, Pl. IV.), a section through a young tentacle bud, the boundaries of all of the cells except the nematocysts and gland cells becomes lost so that the whole layer constitutes a syncytium. The cytoplasm is uniformly granular throughout. The nuclei are scattered through the cytoplasm, but still indicate by their positions the fact that only definite cell walls are wanting in order that the usual cell structure should be apparent. At the internal boundary of the entoderm there is distinguishable a layer of very fine muscle fibers. When compared with the size of the nuclei and nematocyst cells, these fibers are seen to be several times less than the diameter of normal muscle fibers of this species of actinian. Since in the fully developed *Aiptasia* muscle fibers exist both as processes from epithelio-muscle cells and as nearly the entire component of "muscle cells" in which the cytoplasmic cell body is reduced to a small mass containing the nucleus, it is impossible in this case to determine whether we have to do with fibers of the first or of the second of the two above-mentioned kinds. In other words the fibers in question may be in the process of becoming differentiated from epithelial cells, or they may be arising through the division of previously existing muscle cells of the second type. At this stage there is no indication of interstitial cells or of developing gland cells in the ectoderm.

In the endodermal layer of the same section there is exhibited a similar condition of the tissue: Cell walls are entirely wanting; the nuclei, however, by their position indicate a potential cellular arrangement of the cytoplasm. A particularly striking feature as regards this layer is brought out by a comparison of the figure under discussion and Fig. 7, Pl. II. In the last-mentioned figure the endoderm, throughout most of its extent, is considerably thicker than the ectoderm, while in the new tissue the former is generally so thin that the single zoöxanthellæ are greater in diameter than the layer of cytoplasm making up the endoderm and consequently they cause a prominence extending into the cavity of the tentacle wherever they are situated. In some few instances the zoöxanthellæ are placed one above the other, and n such cases there is either a generally thickened area of the cytoplasm or else a cylindrical prominence, as is seen on one side of the figure. All cellular differentiation is entirely lacking in the endoderm. At no place about the circumference of this layer is there any indication of the layer of endodermal (circular) muscles, which are in normal *Aiptasia* tissues, when seen in transverse sections of the animal, much more prominent than the ectodermal muscles.

At the base of the fragment where the rearrangement of the materials is proceeding at a relatively slow rate there seems never to be a breaking down of the tissues comparable to that just described. The mesogleal layer becomes thinned out progressively so that at first it is of its usual thickness on one side of the fragment—that side originally opposite the point where the fragment was torn off from the parent animal—while on the opposite, more actively growing, side it has become relatively thin (Fig. 13, Pl. III.). The cellular layers retain practically their original characteristic appearance, although, as is shown in Fig. 15, Pl. III., the ectoderm on the side where the new growth is the most rapid, *i. e.*, where there are no old mesenteries in the figure, is much lower than on the opposite side.

When in the later development the cellular elements of the ectoderm and endoderm become delimited by the appearance of cell walls it is noticeable that all of the cells show at first an embryonic character in that they are relatively wide in proportion to their height (compare Figs. 10 and 11 with the old part of the tissue in Fig. 14). In the ectoderm of the column wall in Figs. 10 and 11, and more especially in the lining of the stomodeum of this individual, there is a very marked paucity of the nematocysts and gland cells, so that it is apparent that there has not been an increase in these cellular elements to keep pace with the increase in the epithelial cells. In the older embryo from which the series of figures from 15 to 18 was taken the nematocysts and gland cells have reached nearly their normal number and distribution throughout the newly formed tissues.

In the sections shown in Figs. 10 and 11 the endoderm cells are in general broader than the ectoderm cells and not generally of greater height. In the older tissue (Fig. 18) there are no apparent cell boundaries in the endoderm and the zoöxanthellæ are much more numerous.

## Summary.

1. The change in the form of a laceration piece, leading up to the acquisition of the typical actinian shape, takes place through the upgrowth of the tissue about the orifice where it was torn off from the parent.

2. The permanent mesenteries arise as new growths in the undifferentiated tissues of the oral end of the laceration piece.

3. The first twelve mesenteries do not appear in the sequence followed by those in egg embryos.

4. As development goes on the old mesenteries—those brought over from the parent when the fragment was torn off—become restricted to a proportionately shorter and shorter part of the base of the young actinian until they are finally entirely resorbed.

5. The mesenteric filaments are formed, just as in egg embryos, from a downgrowth of the ectodermal lining of the stomodeum. Their trilobed condition arises through the differentiation of this tissue.

6. The tissues of the most actively growing part of a laceration piece become very thin; the ectoderm and the entoderm lose all apparent cell boundaries; the mesoglea arises as a direct continuation of that present in the older tissues.

7. The newly formed tissues contain a very small number of gland cells and nematocysts. These two types of cells are developed to the usual number after the tissue relations have bebecome stable.

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## Explanation of Plates.

All the figures were drawn from specimens of *A. pallida;* those of the entire laceration pieces from specimens grown in the laboratory; the sections from specimens obtained at Beaufort, N. C.

The ectoderm is stippled in all the figures, the endoderm is distinguished by the presence of the zoöxanthellæ, the mesoglea has been represented by either a single line in the actively growing parts of the embryo or by a clear space between the cellular layers in the older tissues.

### Plate I.

The figures on this plate represent a series of stages in the development of a laceration piece, from the time of its separation from the parent until it has assumed the form of a typical young actinian.

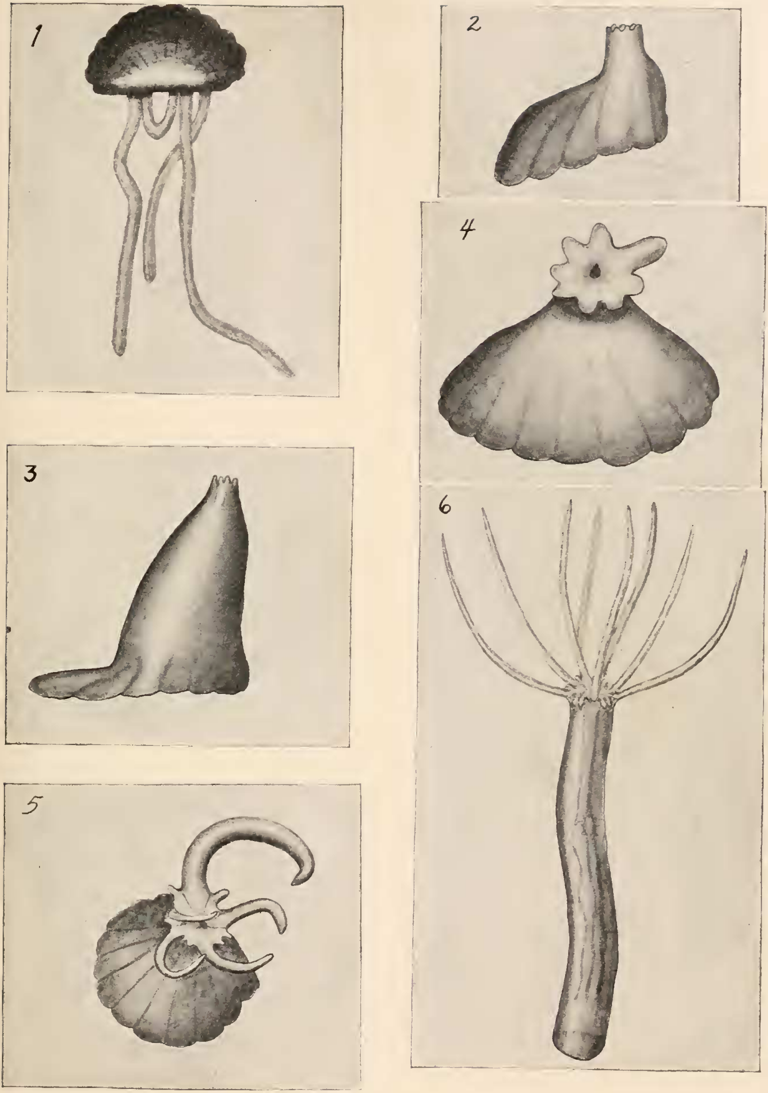
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PLATE II.

FIG. 7. A section through a laceration piece soon after its separation from the parent.

FIG. 8. Part of a vertical section through a young bud to show the formation of the stomodeum.

FIG. 9. Part of a longitudinal section through a young laceration embryo, such as is shown in Fig. 4, Plate I., to show the downgrowth of the ectodermal lining of the stomodeum to form the mesenteric filaments.

FIGS. 10, 11, 12 (with FIGS. 13 and 14, PLATE III.). A series of sections at different levels taken through the same embryo to show the relation of the new to the old mesenteries. (The orientation is the same in all the figures.)

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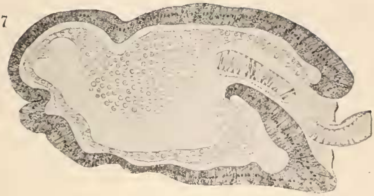

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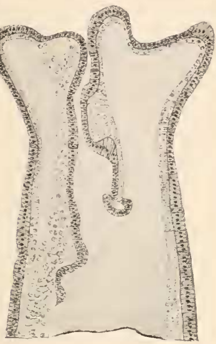

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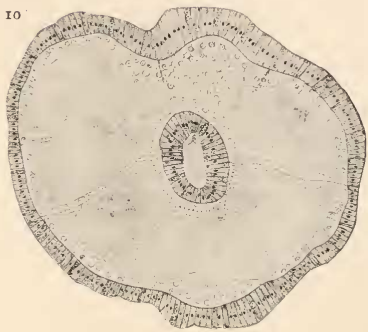

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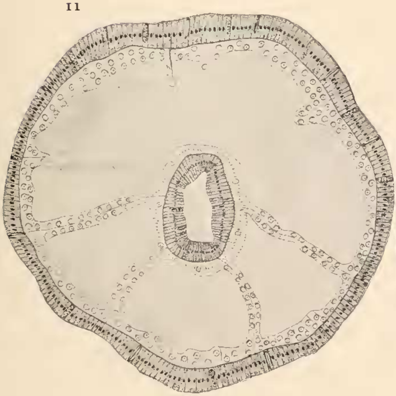

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